Risks of Metolachlor Use to Federally Listed Endangered Barton Springs Salamander (Eurycea sosorum)

May 2007

Risks of Metolachlor Use to Federally Listed Endangered Barton Springs Salamander
(Eurycea sosorum)

Pesticide Effects Determination

Environmental Fate and Effects Division
Office of Pesticide Programs
Washington, D.C. 20460

May 3, 2007

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1.0 Executive Summary

This ecological risk assessment evaluates the potential for the use of the herbicide metolachlor to affect the Barton Springs salamander *(Eurycea sosorum).* The Barton Springs salamander was Federally listed as an endangered species on May 30, 1997. No critical habitat was designated. The salamander has an extremely limited geographic range, inhabiting only four freshwater springs, known as the Barton Springs complex, in Austin, Texas. The salamanders are aquatic throughout their entire lifespan, and in addition to the springs, their habitat includes fractures in the karst system which supplies ground water to the springs (USFWS 2005). The distance they travel into the fractures, and the specific habitat use of this area is unknown. This assessment is one of a series of ecological risk assessments developed consistent with the settlement for the court case *Center for Biological Diversity and Save Our Springs Alliance v. Leavitt, No. 1:04CV00126-CKK (filed January 26, 2004).*

Metolachlor (PC#108801) is a pre-plant, pre-emergence herbicide, currently registered for use only on agricultural crops. As originally registered, metolachlor (PC#108801) is a racemic mixture of *r*- and *s*-enantiomers. Of these enantiomers, the *s*-enantiomer has been shown to be more biologically active in plants, and an enriched technical product is registered separately (PC#108800) as S-metolachlor. This assessment addresses use of racemic metolachlor, although toxicity data from both chemicals has been included.

Metolachlor is persistent and mobile in soil. It is highly persistent in water and has been detected extensively in both surface and ground water. It is a biosynthesis inhibitor, absorbed through the roots and the shoots of the plant. Metolachlor is slightly to moderately toxic to freshwater fish, amphibians, and freshwater invertebrates on an acute basis (LC_{50}s 1.1-26 mg/L). Toxicity to aquatic plants (EC_{50}s) ranges from 0.008–1.2 mg/L. Based on standard environmental fate tests, metolachlor has two major[1] degradates, metolachlor oxanilic acid (OA) and metolachlor ethane sulfonic acid (ESA). Both degradates are less toxic than the parent metolachlor and are considered in the risk assessment.

The salamander is neotenic (aquatic throughout its life history), thus the assessment focuses on the components of the aquatic system, including aquatic plants, invertebrates, and the salamander itself. Although terrestrial plants serve important functions in riparian systems, analysis of metolachlor uses in the Barton Springs action area showed no terrestrial plants near the springs were likely to be exposed to metolachlor. No metolachlor is used in Zilker Park, where the springs are located, and the closest use sites are 5-10 miles away from the spring, so spray drift is unlikely. Based on analysis of land use and pesticide use data, there appears to be little or no use of metolachlor in the land areas contributing to the Barton Springs Segment of the Edwards Aquifer (BSSEA), the sole ground water source for the springs, and primary pesticide exposure route for the salamander.

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[1] Defined as ≥10% of applied.

Although there are limited agricultural land uses in the area contributing to the BSSEA, they do exist, and there is potential for metolachlor to be introduced to the springs from those uses. Based on land use and pesticide usage data, it appears the only potential use of metolachlor in the area contributing water to the springs is on sorghum grown for hay. USGS monitoring detected metolachlor in the springs, ground water, and the surface water in the Barton Springs area. However, it was detected infrequently (6% of samples) and generally at or near the analytical limit of detection (LOD) of 0.013 μg/L. Peak modeled concentrations in the springs ranged from 11.8-18.0 μg/L based the meadow/hay scenario specifically developed for the Barton Springs area.

The Environmental Fate and Effects Division (EFED) evaluated direct effects (survival, reproduction, and growth) of metolachlor on the Barton Springs salamander, and indirect effects (reduction of prey base, habitat modification) on the ecosystem which supports the salamander. Effects determinations were made in accordance with procedures described in the Agency's Overview Document (U.S. EPA 2004), using the best available data. Based on this analysis EFED finds that the use of metolachlor, in accordance with existing approved labels, may effect but is not likely to adversely affect the Barton Springs salamander. Assessment endpoints and the basis of determination for each endpoint evaluated are shown in Table 1.

Table 1 Effects Determination for Metolachlor

Assessment Endpoint	Effects determination	Basis for Determination
Direct Effects		
Survival, growth, and reproduction of Barton Springs salamander	No effect	No LOC exceedences for surrogate taxa (freshwater fish) representing Barton Springs salamander.
Indirect Effects		
Reduction of prey (*i.e.,* freshwater invertebrates)	May affect Not likely to adversely affect (Discountable)	No acute LOC exceedences for freshwater invertebrates. Chronic LOC exceedence at highest peak modeled concentrations, but not any others. Effects noted in study used to establish assessment endpoint occurred at concentrations an order of magnitude higher than modeled concentrations. Modeled concentrations are 5 orders of magnitude higher than monitored concentrations.
Degradation of habitat and/or primary productivity (*i.e.,* aquatic plants)	May affect Not likely to adversely affect (Discountable)	LOC exceedences at modeled peak EEC for most sensitive freshwater plant species (green alga), but not for any other freshwater plant species for which data was available. No exceedences based on monitored concentrations.

2.0 Problem Formulation

2.1 Purpose

This ecological risk assessment has been conducted consistent with the settlement of the court case *Center for Biological Diversity and Save Our Springs Alliance v. Leavitt, No. 1:04CV00126-CKK (filed January 26, 2004).* The purpose of this ecological risk assessment is to determine if the registration of the herbicide metolachlor (PC 108801) could affect the Barton Springs salamander *(Eurycea sosorum),* implementing the Environmental Protection Agency's (the Agency) responsibility as directed in Section 7(a)(2) of the Endangered Species Act (ESA). The Barton Springs salamander was Federally listed as an endangered species on May 30, 1997 (62 FR 23377-23392) by the U.S. Fish and Wildlife Service (USFWS or the Service). No critical habitat has been designated for this species.

In this assessment, direct and indirect effects to the survival, growth, and reproduction of the Barton Springs salamander are evaluated in accordance with methodologies described in the Agency's Overview Document (U.S. EPA 2004).

As part of the "effects determination", the Agency reaches one of the following three conclusions regarding the potential for metolachlor to affect the Barton Springs salamander:

- "No effect";
- "May affect, but not likely to adversely affect"; or
- "May affect, likely to adversely affect".

If the results of the screening-level assessment show that pre-established levels of concern (LOCs) are not exceeded for direct effects on the Barton Springs salamander (U.S. EPA 2004), and no indirect effects are expected (*e.g.,* degradation of habitat or reduction of prey availability), a "no effect" determination is made. Exposure estimates are made based on both the potential and reported use of metolachlor within the action area. If, however, indirect effects are anticipated and/or estimated exposure exceeds the LOCs for direct effects, the Agency concludes a preliminary "may affect" determination for the Barton Springs salamander.

If a determination is made that use of metolachlor within the action area "may affect" the Barton Springs salamander, additional information is considered to refine the potential for exposure and evaluate the anticipated effects. The Agency uses the best available information to determine if the registered uses are "not likely to adversely affect (NLAA)" or "likely to adversely affect (LAA)" the Barton Springs salamander.

2.2 Scope

The end result of the Agency's pesticide registration process is an approved product label. The label is a legal document that stipulates how and on what use sites a given pesticide may be used. Product labels (also known as end-use labels) describe the formulation type, acceptable methods of application, approved use sites (*i.e.,* specific crops), and any restrictions on how applications may be conducted. Thus, the use or potential use of metolachlor in accordance with the approved product labels is the "action" being assessed. The majority of guideline toxicity data available pertains to the active ingredient ("ai"), and the effects of the active ingredient form the basis of the evaluation. When formulation-based toxicity data are available, they are considered in the assessment.

Metolachlor (PC 108801, CAS Registry #s 51218-45-2 and 87392-12-9), is an herbicide currently registered in the U.S. for agricultural uses only, on a limited number of crops. These crops include beans, corn, cotton, legume vegetables, potatoes, safflower and sorghum. Metolachlor has two major degradates, (metolachlor ESA and metolachlor OA) that have been detected in both surface and ground water. Both of these degradates are considered in the assessment. This assessment does not evaluate S-metolachlor (PC 108800), which is a separate registration.

The current registration for metolachlor allows for use nationwide, thus the action area for the entire registration would include areas throughout the United States and its territories. However, because this ecological risk assessment is species specific for the Barton Springs salamander (BSS), the BSS-metolachlor action area is defined as the locations where metolachlor, if used in accordance with label instructions, might reasonably be expected to be transported to a location where the salamander and/or key components of its supporting ecosystem might be exposed to it (*i.e.,*completed exposure pathways). Further discussion of the action area for the Barton Springs salamander is provided in Section 2.6.

2.3 Previous Assessments

2.3.1 Metolachlor

The Agency has completed other assessments on metolachlor, including an evaluation of the potential effects on 26 Evolutionarily Significant Units (ESUs) of listed salmonids in the Pacific Northwest (PNW) (U.S. EPA 2006a) and the 1993 Re-registration (RED) document (U.S. EPA 1995). The 1995 metolachlor ecological risk assessment for the RED identified an exceedence of the endangered species risk level of concern (LOC) for fish, based on runoff into a shallow (6-inch) water body from roadside use (roadside ditch). In the years following the re-registration of metolachlor, EFED incorporated the use of more advanced exposure models into the risk assessment process. Other, more mechanistic modeling approaches have replaced the roadside ditch.

The 2006 evaluation concerning the salmonids was more comprehensive than the RED. It incorporated the updated exposure models, and evaluated both direct and indirect effects, as required by the Endangered Species Act (ESA). The salmonid assessment was conducted in accordance with methods described in the Overview Document It considered the effects of metolachlor on primary productivity in aquatic systems and evaluated the potential impact of metolachlor on riparian vegetation in addition to considering the direct effects to fish and invertebrates (U.S. EPA 2006a). The 2006 salmonid assessment incorporated the methodologies described in the Overview Document (U.S. EPA 2004), which are the same basic methods used in this assessment. In summary, the salmonid assessment found use of metolachlor as registered:

- Would have no (direct) effect on salmonids (survival, growth or reproduction)
- Was not likely to adversely affect salmonid prey
- Was not likely to adversely affect aquatic plants, and
- Was not likely to adversely affect riparian vegetation.

An ecological and human health risk assessment conducted by the U.S. Geological Survey for the Lower Missouri River focused on the potential for metolachlor to affect aquatic plants (Fairchild *et al.,* 1999). The assessment used toxicity data for an algal species (*Selenastrum capricornutum)* and an aquatic vascular plant *(Lemna minor)* compared to monitored and modeled metolachlor concentrations. While modeled concentrations were expected to result in effects to aquatic plants, highest monitored values were below toxic thresholds. The assessment concluded "…that adverse impact of herbicides on non-target plant communities of the Lower Missouri River are unlikely."

2.3.2 Barton Springs Salamander

The Agency has also completed (U.S. EPA 2006b) an ecological risk assessment evaluating the potential effects of another herbicide, atrazine, on the Barton Springs Salamander. The atrazine assessment was conducted consistent with the settlement for the court case *Center for Biological Diversity and Save Our Springs Alliance v. Leavitt, No. 1:04CV00126-CKK (filed January 26, 2004).* Conclusions regarding atrazine use in the action area were that it:

- Would have no (direct) effect on the Barton Springs salamander (survival, growth or reproduction),
- Was not likely to adversely affect salamander prey, and
- Was not likely to adversely affect aquatic plants.

2.4 Stressor Source and Distribution

2.4.1 Environmental Fate and Transport Assessment

Acceptable environmental fate data indicate that parent metolachlor appears to be moderately persistent to persistent in soil. It ranges from mobile to highly mobile in different soils and has been detected extensively in surface water and ground water. Metolachlor degradation appears to be dependent on both microbially mediated (aerobic soil metabolism $t_{1/2}$ = 66 days, 37.8 days, 37.8 days, 14.9 days, 13.9 days, and 50.3 days, anaerobic soil metabolism $t_{1/2}$ =81 days) and abiotic processes (photodegradation in water $t_{1/2}$ = 70 days under natural sunlight and photodegradation on soil $t_{1/2}$ = 8 days under natural sunlight).

The major degradates of metolachlor were initially identified as CGA-51202 (metolachlor oxanilic acid (OA)), CGA-50720, CGA-41638, CGA-37735, and CGA-13656. Subsequent studies also identified CGA-354743 (metolachlor ethane sulfonic acid (ESA)). Of these major degradates, metolachlor ESA and metolachlor OA have been identified in both ground water and surface water. Depending on the soil (*i.e.,* organic matter content), metolachlor has the potential to range from a moderately mobile to a highly mobile compound with K_d values ranging from 0.11 to 44.8, and K_{oc} values ranging from 21.6 to 367.

Field dissipation studies indicate that metolachlor is persistent in surface soil, with half lives ranging from 7 to 292 days in the upper six inch soil layer, depending on geographic location. Metolachlor was reportedly detected as deep as the 36 to 48 inch soil layer in some studies. Metolachlor OA (CGA-51202), was detected (0.11 ppm) as deep as the 30-36 inch soil depth (MRID 41335701); CGA-40172 was detected as deep as the 36-48 inches (MRID 41309802); CGA-40919 was detected in the 36-48 inch depth (0.21 ppm in MRID 41309802); and CGA-50720 was not detected (LOD = 0.07 ppm) in any soil segment at any interval.

2.4.2 Mechanism of Action

Metolachlor is a biosysnthesis inhibitor. It is absorbed by the roots and shoots of the plant and translocates in the plant (www.syngentacropprotection-us.com/prod/herbicide/dualiimagnum). Germinating monocots primarily absorb metolachlor through the shoot just above the seed, and germinating dicots absorb at the shoot and the roots (Zimdahl 1993). Metolachlor may be active in the soil for several months following application.

A single specific biochemical target of metolachlor and other chloroacetamide herbicides has not been defined and it appears the chemicals may act via multiple pathways. Alkylation appears to be important in phytotoxicity (Jablonkai 2003) and lipophilicity has been correlated with algal reproduction effects (Junghans *et al.* 2003). Covalent inhibition of coenzyme A elongation (Schmalfuss *et al.* 2000) and covalent inhibition of very-long-chain fatty acid synthesis via chalcone synthase have been proposed as mechanisms of action in terrestrial plants (Eckermann *et al.* 2003). Inhibition of protein biosynthesis has also been proposed as a mechanism of action in plants (Pillai *et al.* 1979). Several proposed mechanisms of action in plants involve irreversible, covalent binding to cysteine residues. Consistent with cysteine reactivity, glutathione S-transerfase has been shown to be important in detoxifying chloroacetanilide herbicides in tolerant plants (Rossini *et al.* 1998).

2.4.3 Use Characterization

An analysis of available usage and land cover information, including extensive discussions with local experts in the fields of agriculture and soil science, was completed to determine which metolachlor use sites are likely to be present in the area contributing surface or ground water to Barton Springs.

Use and usage information is critical to the development of appropriate modeling scenarios and to selection of the appropriate model inputs for exposure estimates. The Agency's Office of Pesticide Programs Biological and Economic Analysis Division (OPP/BEAD) provided an analysis of both national and local use information for metolachlor (Kaul *et al*., 2005, Zinn and Jones, 2006, Kaul, *et al*., 2006). State level usage data, obtained from USDA-NASS[2] and EPA proprietary data[3] sources were averaged together over the years 2000 to 2004 to calculate average annual usage statistics by state and crop for metolachlor, including pounds of active ingredient applied, percent of crop treated, number of applications per acre, application rate per acre, and base acres treated.

[2] United States Depart of Agriculture (USDA), National Agricultural Statistics Service (NASS) Chemical Use Reports provide summary pesticide usage statistics for select agricultural use sites by chemical, crop and state. See http://www.usda.gov/nass/pubs/estindx1.htm#agchem.

[3] US EPA proprietary usage databases provide estimates of pesticide usage for select agricultural use sties by chemical, crop and state.

Because no reliable county level usage data is available for Texas, average annual pounds applied and acres treated by county were calculated by apportioning the estimated state level usage to counties based on the proportion of total state acres grown of each crop in each county. The most recently available acreage data was obtained from USDA's 2002 Census of Agriculture. Estimates of the percent of each crop treated, the number of applications and the application rate in each county are assumed to be the same as the state level estimates. Apportioning the usage in this manner may underestimate or overestimate the actual usage in a particular county.

In this analysis, the Agency gathered information on the agricultural uses of metolachlor in the three counties (Hays, Travis, and Blanco) located within or adjacent to the action area defined for the Barton Springs salamander described in Section 2.6). Information was available on crops for which metolachlor is registered, estimated amounts of metolachlor used by county, application rates, method of application, application timing, and intervals between applications. Usage information is critical in determining which uses should be modeled, while the application methods, intervals, and timing are critical model inputs for estimating metolachlor exposure.

An evaluation of usage information was completed to determine whether any or all of the area defined by the BSSEA should be included in the action area. Current end-use[4] labels and local use information were reviewed to determine which metolachlor uses could possibly be present within the defined area. Local land cover data (City of Austin, 2003a and b; USGS, 2003) was analyzed and interviews with the local agricultural sector (Davis, 2006; Garcia, 2006; Perez, 2006; see Appendix C for more detail) were conducted to refine the list of potential metolachlor use sites. In Travis, Hays, and Blanco counties, crops grown for which metolachlor is registered include cotton, grasses grown for seed, beans, corn, legume vegetables, and sorghum (Kaul and Carter 2005). In the process of scenario development, it was found that all commercial crops grown in the three counties of interest are planted east of Interstate 35 (I-35), which is out of any of the zones which contribute surface or ground water to the springs (SRC 2006). However, this analysis indicated that there are some meadows where sorghum could be grown for hay within the area that contributes water to the springs, although it appears unlikely. Metolachlor could legally be used on these sites, so this use was modeled to ensure a comprehensive assessment.

[4] Technical labels also exist, which may include crops not listed on the end use labels. Technical products are used to make formulated end use products. Because these technicals are cannot be applied directly, use sites on these labels are not considered a part of the Federal action.

2.5 Assessed Species

The Barton Springs salamander is aquatic throughout its entire life cycle. Members of the Plethodontidae Family (lungless salamanders), they retain their gills, become sexually mature, and eventually reproduce in freshwater aquatic ecosystems. The best available information indicates the Barton Springs salamander is restricted to the four springs outlets that make up the Barton Springs complex (Figure 1), located in Zilker Park near downtown Austin, Texas. As such, this species has one of the smallest ranges of any vertebrate species in North America (Chippindale 1993). The Barton Springs segment of the Edwards Aquifer and its contributing zone supply all of the water in the springs that make up the Barton Springs complex. Flows of clean spring water are essential to maintaining well-oxygenated water necessary for salamander respiration and survival.

The subterranean component of the Barton Spring salamander's habitat may provide a location for reproduction (USFWS, 2005); however, little is known about the reproductive biology of the Barton Springs salamander in the wild. It appears that salamanders can reproduce year-round, based on observations of gravid females, eggs, and larvae throughout the year in Barton Springs (USFWS 2005). Survey results indicate Barton Springs salamanders prefer areas near the spring outflows, with clean, loose substrate for cover, but they may also be associated with aquatic plants, especially moss. In addition to providing cover, moss and other aquatic plants harbor a variety and abundance of the salamander's prey, freshwater invertebrates. Based on available information, both adults and juveniles eat freshwater invertebrates (USFWS 2005).

Further information on the status and life history of the Barton Springs salamander is provided in Appendix A.

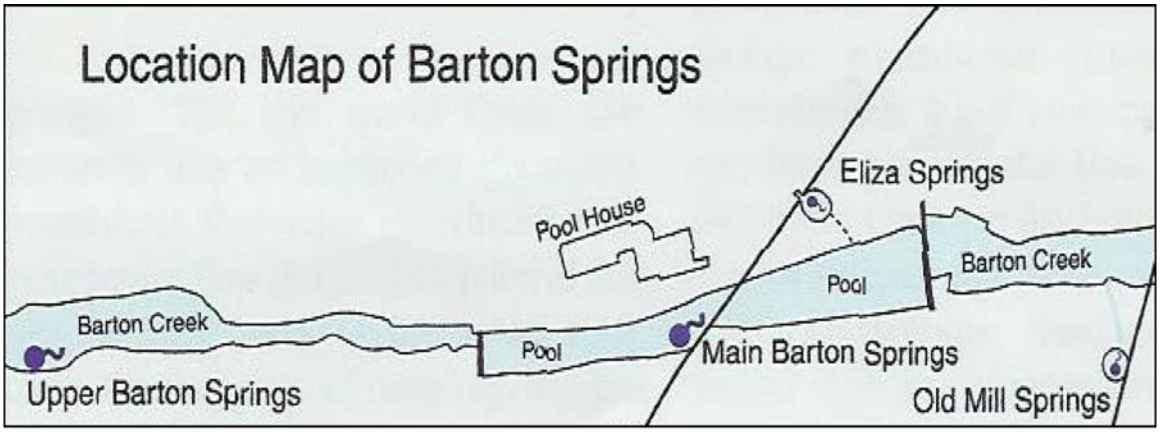

Diagram from Hauwert et al., Barton Springs Edwards Aquifer Conservation District Report

Figure 1 Location Map of Barton Springs

Figure 2 Aerial Photo of Barton Springs

2.6 Action Area

It is recognized by the Agency that the overall action area for the national registration of metolachlor includes any locations where registered uses might result in ecological effects. However, the scope of this assessment limits consideration of the overall action area to locations of those crops applicable to the protection of the Barton Springs salamander. Thus, the BSS-metolachlor action area is defined largely by the hydrogeologic framework of Barton Springs. Deriving the geographical extent of this portion of the action area is the product of consideration of the types of effects metolachlor may be expected to have on the environment, the concentrations of metolachlor that are associated with those effects, and the best available information concerning the use of metolachlor and its fate and transport within the Barton Springs area.

Unlike exposure pathways for most aquatic organisms, where stressors are transported via surface water to the receptor within a defined watershed, the habitat of the Barton Springs salamander is almost completely ground water driven. Runoff from treated fields, transported through the fractured limestone (karst) of the Edwards Aquifer, is the principal route of exposure for the salamander (U.S. EPA 2006b). Thus, the action area for this assessment is defined by those areas within the hydrogeologic "watershed," including the Barton Springs Segment of the Edwards Aquifer and the Contributing Zone (BSSEA), that supply water to the four springs (Main Barton Springs, Eliza Springs, Old Mill Springs, and Upper Barton Springs; see Figure 1 and Figure 2) occupied by the salamanders (USFWS 2005). During high flow conditions, surface water flow from Barton Creek may enter the pool if it overtops the dam at the upper end of the pool. Any pesticide used in the land areas contributing to the ground water in the Barton Springs segment of the aquifer or to the surface water in Barton Creek could potentially be transported to the springs.

Flow to the Barton Springs is controlled by the geology and hydrogeology of the BSSEA. Numerous geological and ground water studies (Slade *et al*., 1986, Hauwert *et al.,* 2004) have been conducted to define the extent of the area contributing to the Barton Springs and characterize the flow within the system. The BSSEA is a 354 square mile area, comprised of four hydrogeologic zones. These are, from west to east, the Contributing Zone, the Recharge Zone, the Transition Zone, and the Artesian Zone. Of these zones, the Contributing and Recharge Zones have the greatest and most direct influence on Barton Springs. There is evidence that the Transition Zone has some limited input into the Barton Springs, while the Artesian Zone contributes no subsurface flow to the springs (Slade *et al.,* 1985, Hauwert *et al.,* 2004). The BSSEA is characterized as a karst system permitting relatively rapid transit of ground water, with velocities along the dominant flow path of 1-5 miles/day, depending on ground water flow conditions (USFWS 2005) particularly within the fracture portions. Based on dye tracer studies, pesticides applied within the recharge and contributing zones could potentially be present in the water of the springs on a time scale of days to weeks (Hauwert *et al*., 2004)

Spray drift and/or long-range atmospheric transport of pesticides could potentially contribute to concentrations in the aquatic habitat used by the salamander. Given the physico-chemical profile for metolachlor and the fact that metolachlor has been detected in both air and rainfall samples, the potential for long range transport from outside the area defined by the BSSEA cannot be precluded, but is not expected to approach concentrations in runoff predicted by modeling. Metolachlor introduced to the ground water system via atmospheric deposition or other environmental processes not specifically accounted for in the assessment is addressed by evaluation of the monitoring data, and incorporation of a background concentration into the exposure analysis.

Thus, the action area for metolachlor as it relates to the Barton Springs salamander (the "BSS-metolachlor action area") is defined by the Contributing Zone, Recharge Zone, and Transition Zone within the BSSEA (Figure 3).

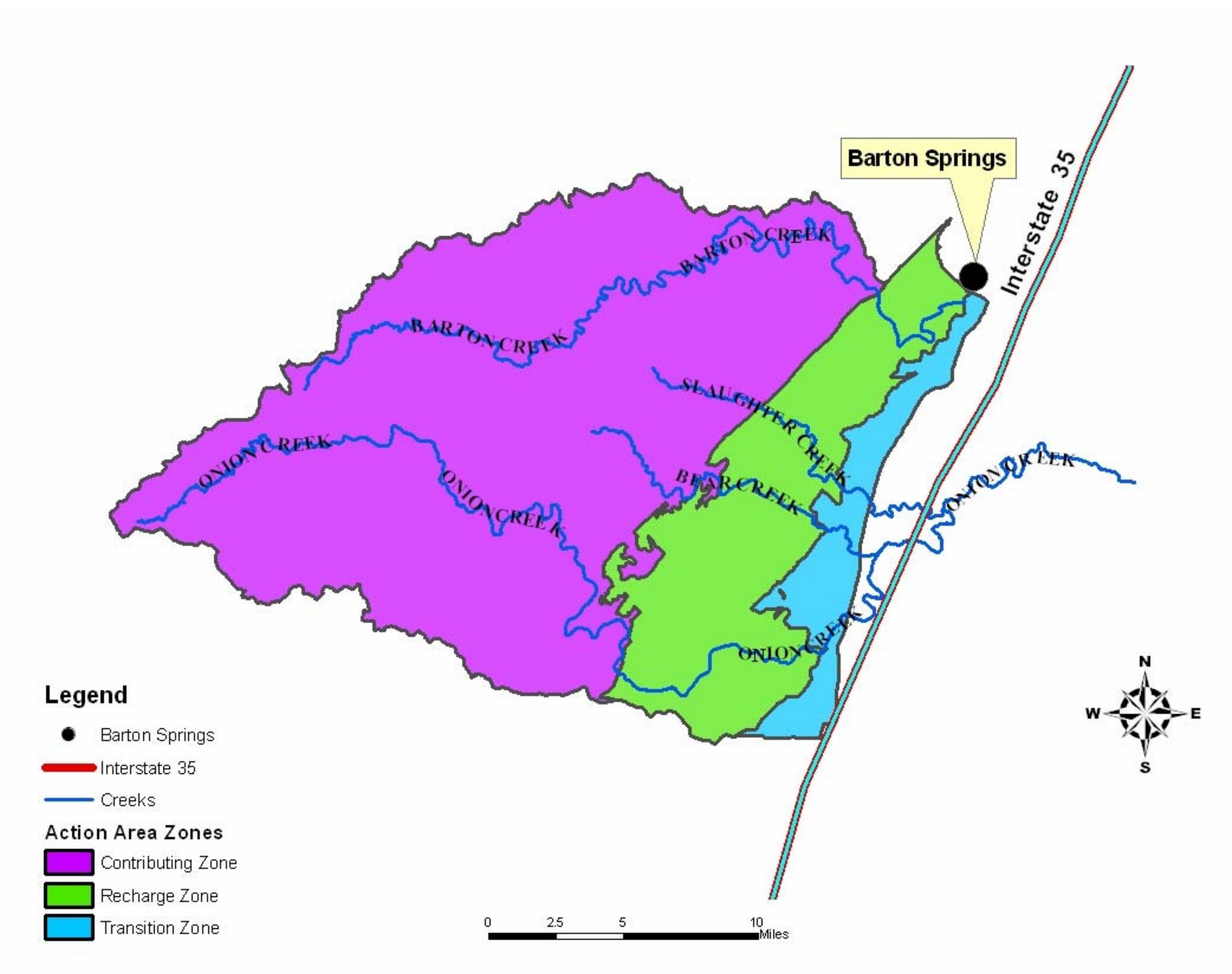

Figure 3 Metolachlor Action Area

2.7 *Assessment Endpoints and Measures of Ecological Effect*

Assessment endpoints are defined as "explicit expressions of the actual environmental value that is to be protected."[5] Selection of the assessment endpoints is based on valued entities (*i.e.,* Barton Springs salamanders), the ecosystems potentially at risk (*i.e,* Barton Springs), the migration pathways of metolachlor (*i.e.,*ground water and surface water tranport), and the routes by which ecological receptors are exposed to metolachlor-related contamination (*i.e.,* direct contact in aqueous medium).

Assessment endpoints for the Barton Springs salamander include direct toxic effects on the survival, reproduction, and growth of the salamander itself, as well as indirect effects, such as reduction of the prey base and/or modification of its habitat. Each assessment endpoint requires one or more "measures of ecological effect," which are defined as changes in the attributes of an assessment endpoint itself or changes in a surrogate entity or attribute in response to exposure to a pesticide. Measures of ecological effect are evaluated based on acute and chronic toxicity information from registrant-submitted guideline tests, and data from open literature which meets specific acceptance criteria[6]. Guideline test are performed on a limited number of organisms, which serve as surrogates for other types of organisms expected to have similar responses. Open literature data may expand the number of organisms for which toxicity data are available, but these tests may or may not have been conducted in accordance with standardized protocols and are often not directly comparable to the guideline tests. EFED guidance (U.S. EPA 2004) specifies that, in absence of data from more closely related species, bird toxicity data is used for terrestrial-phase amphibians and fish data are used for aquatic-phase amphibians. Species-sensitivity distributions are not well understood, thus to provide a conservative estimate of risk EFED uses the most sensitive organism in the representative phylogenic class. Barton Springs salamanders are neotenic (retain gills throughout their lives) and are considered aquatic-phase amphibians. No species-specific toxicity data were available at the time of this risk assessment. Therefore, fish data and/or other amphibian data are used as surrogates for the Barton Springs salamander, in accordance with guidance specified in the Agency's Overview Document (U.S. EPA 2004).

Table 2 Summary of Assessment Endpoints and Measures of Ecological Effect

Assessment Endpoint	Measures of Ecological Effect[7]
1. Survival, growth, and reproduction of Barton Springs salamander individuals via direct effects	1a. Bluegill sunfish acute LC_{50} 1b. Fathead minnow chronic NOAEC
2. Survival, growth, and reproduction of Barton Springs salamander individuals via indirect effects on prey (*i.e.,* freshwater invertebrates)	2a. Water flea acute EC_{50} 2b. Water flea chronic NOAEC
3. Survival, growth, and reproduction of Barton Springs salamander individuals via indirect effects on habitat and/or primary productivity (*i.e.,* aquatic plant community)	3a. Vascular plant (duckweed) acute EC_{50} 3b. Non-vascular plant (freshwater algae) acute EC_{50}

[5] From U.S. EPA (1992). *Framework for Ecological Risk Assessment.* EPA/630/R-92/001.
[6] For exact guidelines, see the "Overview Document, "(U.S EPA 2004)
[7] All toxicity data reviewed for this assessment are included in Appendix B.

2.8 Conceptual Model

2.8.1 Risk Hypotheses

Risk hypotheses are specific assumptions about potential adverse effects (*i.e.,* changes in assessment endpoints) and may be based on theory and logic, empirical data, mathematical models, or probability models (U.S. EPA, 1998). For this assessment, the risk is stressor-linked, where the stressor is intentional release of metolachlor to the environment by application on agricultural crops. Based on the results of previous ecological risk assessments regarding metolachlor, the following risk hypotheses are evaluated in this endangered species assessment:

- Metolachlor in ground water, surface water, and/or runoff from treated areas may directly affect Barton Springs salamanders by causing mortality or adversely affecting growth or fecundity;
- Metolachlor in ground water, surface water, and/or runoff from treated areas may indirectly affect Barton Springs salamanders by reducing or changing the composition of prey populations; and
- Metolachlor in ground water, surface water, and/or runoff from treated areas may indirectly affect Barton Springs salamanders by reducing or changing the composition of the plant community in the springs, thus affecting primary productivity and/or cover.

2.8.2 Diagram

The conceptual model is a graphic representation of the structure of the risk assessment. It specifies the stressor, release mechanisms, abiotic receiving media, biological receptor types, and effects endpoints of potential concern. The conceptual model for the potential effects of metolachlor on the Barton Springs salamander is shown in Figure 4. Exposure routes shown in dashed lines are not quantitatively considered because these exposures are expected to be sufficiently low as not to cause direct or indirect effects to the Barton Springs salamander.

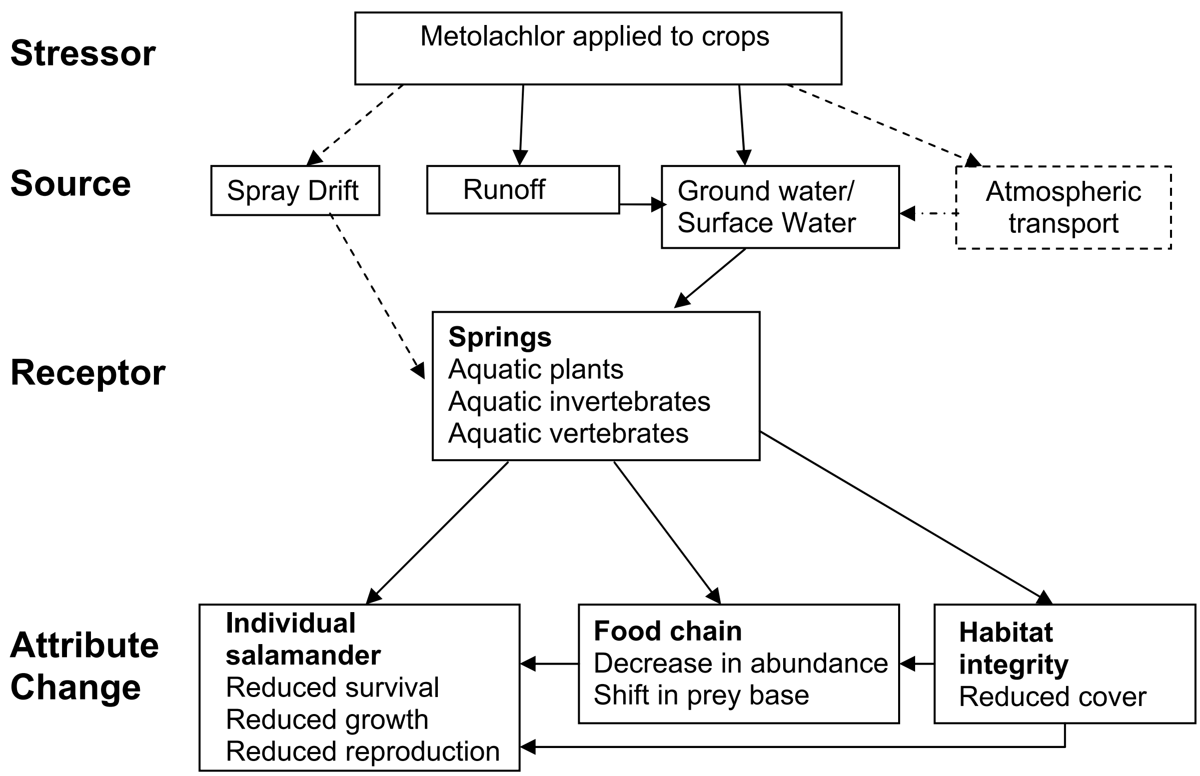

Figure 4 Conceptual Model for Barton Springs Salamander

3.0 Exposure Assessment

The exposure assessment consists of both concentrations based on monitoring data and modeled concentrations. Recent (2000-2004) USGS monitoring data for the surface streams, ground water wells, and the four springs making up the Barton Springs system (Mahler 2005) were available, and are summarized below. Exposure modeling is an application of the standard approach outlined in the Overview Document (U.S. EPA, 2004), modified to reflect the hydrogeologic conditions in the area surrounding Barton Springs. Both sets of exposure estimates are considered in the risk estimation.

3.1 Monitoring Data

USGS provided monitoring data for surface streams, ground water wells, and the four springs making up the Barton Springs system (Mahler, 2005a). In 2000-04, USGS conducted monitoring for an extensive list of pesticides. This study included detection limits an order of magnitude lower than studies conducted earlier (0.013 μg/L, rather than 0.2 μg/L). In addition, the recent data from the USGS targeted single runoff events within the spring systems, with attempts to correlate composition of the sample with the storm hydrograph.

Four springs were included in the USGS analysis: Main Spring, Eliza Spring, Upper Spring, and the Old Mill Spring. These four springs represent the main source of inflow into the Barton Springs pool system, with the Main Spring providing roughly 80% of overall flow.

Data provided by the USGS included some long-term sampling of the Main Springs (1978-2004), and more recent data (2000-2003) for the other springs. Metolachlor was not included in the list of analytes until 1987. In the data from 1987 to 1993, metolachlor was considered a non-detect (LOQ range 0.1-.02 μg/L). Water quality data from 2000-2003 had lower detection limits (LOD range 0.002-0.013 μg/L), but even in this data set, metolachlor was detected in only 1 out of 32 samples, at an estimated concentration of 0.002 μg/L. Monitoring data for Upper Springs, Old Mill Springs, and Eliza Springs were available from 2000 to 2004 (LOD range 0.002-0.013 μg/L). In this data set, metolachlor was only detected once, at an estimated concentration of 0.004 μg/L.

Table 3 Summary of USGS Monitoring Data for Barton Springs Complex

Spring	Range of Sample Dates	# of Samples	# of Detects	Frequency of Detection (%)	Maximum Conc.[1] (µg/L)	Minimum Conc.[2] (µg/L)	Average Conc. (µg/L)
Main	1987-93 2000-04	14 32	0 1	0 3	<0.2 <0.013	<0.1 0.002[3]	NA
Upper	2000-04	14	1	7	<0.013	0.004[3]	NA
Old Mill	2001-03	9	0	0	<0.013	<0.013	NA
Eliza	2001-03	11	0	0	<0.013	<0.002	NA

[1] If there were no quantifiable measurements, this is given as the highest LOD/LOQ in the series
[2] If there were no quantifiable measurements, this is given as the highest LOD/LOQ in the series
[3] Estimated
NA Not applicable, not enough values to average

USGS also had monitoring data for several creeks (Barton Creek, Bear Creek, Onion Creek, Slaughter Creek, and Williamson Creek) in the BSSEA and for ground water wells. Metolachlor was included in the analyte list from 2000-04, with the LOQ generally 0.013 µg/L. Six separate sites were included in the data from Barton Creek, although for three of these sites, metolachlor was not included in the list of analytes. Out of a total of 29 times when it was on the list, it was detected 4 times, at estimated concentrations ranging from 0.003 to 0.010 µg/L. Two of the detections occurred at the sample site in Barton Creek above the springs. Metolachlor was included in the list of analytes for Onion Creek in 2003-2004, but was not detected. Data from Slaughter Creek did not include metolachlor. Monitoring of Williamson Creek included 2 sites, and metolachlor was detected 3 times out of 8, at concentrations ranging from 0.005-0.015 µg/L. Metolachlor was not detected in any of the 8 ground water sites evaluated.

Table 4 Summary of USGS Monitoring Data for Surface and Ground Water in the BSSEA

Water Source	Range of Sample Dates	# of Samples	# of Detects	Frequency of Detection (%)	Max Conc.[1] (µg/L)	Min Conc.[2] (µg/L)	Avg Conc. (µg/L)
Barton Creek (3 sites)	1993-95 2000-04	11 18	0 4	0 22	<0.2 0.010[3]	<0.2 0.003[3]	NA 0.026
Bear Creek (1 site)	1993	1	0	0	<0.2	<0.2	NA
Onion Creek (1 site)	2003-04	2	0	0	<0.013	<0.013	NA
Slaughter Creek	Metolachlor not included on analyte list						
Williamson Creek (2 sites)	2000-04	8	3	38	0.015	0.008	0.028
Ground water Wells (9 sites)	2000-04	36	0	0	<0.013	<0.013	NA

[1] If there were no quantifiable measurements, this is given as the highest LOD/LOQ in the series
[2] If there were no quantifiable measurements, this is given as the highest LOD/LOQ in the series
[3] Estimated

Data from sampling earlier than 2000 generally had higher detections limits (approximately 0.2 µg/L as opposed to 0.01 µg/L), and many of the older data sets did not include metolachlor as an analyte. Detections of metolachlor in later data sets ranged from 0.002 µg/L to 0.010 µg/L. Using the high end of these detections as a benchmark, EFED elected to make the conservative assumption that a background concentration of 0.013 µg/L of metolachlor is present in the waters of the BSSEA supporting Barton Springs.

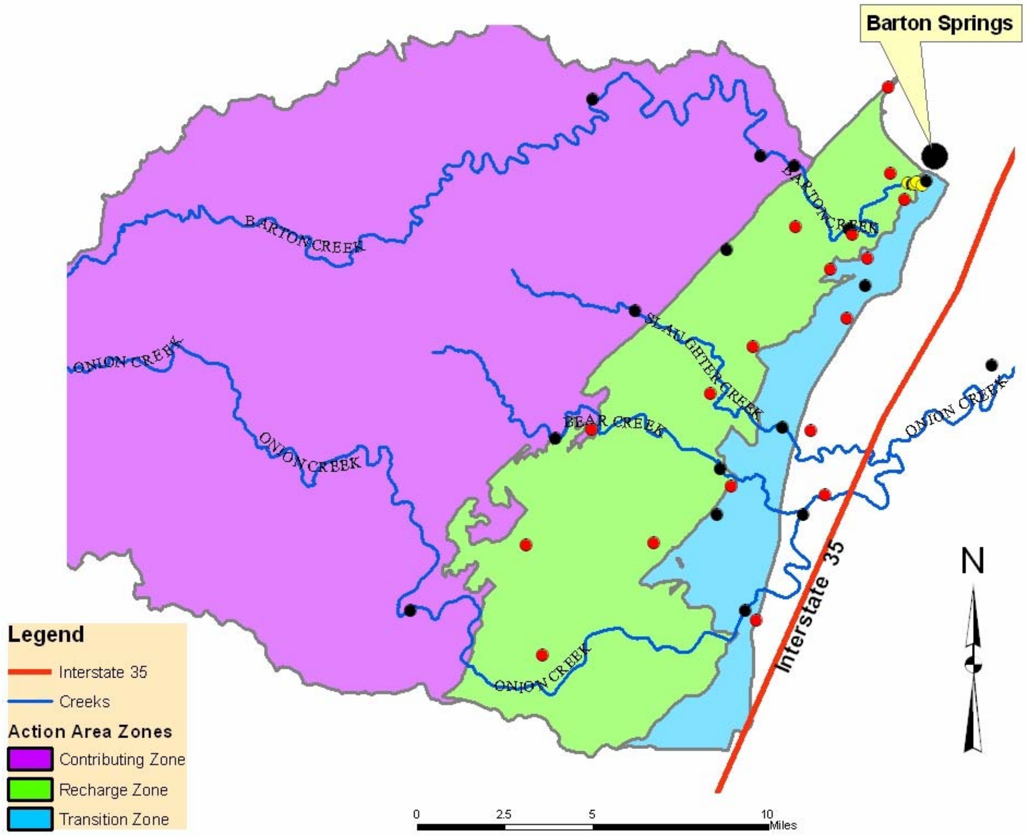

Figure 5 Ground and Surface Water Sampling Sites For Monitoring Data

While of high quality and targeted specifically to the Barton Springs system, the monitoring data may not capture the highest metolachlor concentrations, primarily due to the inherent difficulty of measuring contaminants in runoff events. However, the monitoring data provide a good cross-check to the modeled concentrations and are used in this assessment to establish a background concentration in the ground water.

3.2 *Exposure Estimate Based on Modeling*

The exposure assessment is an application of the standard approach outlined in the Overview Document (U.S. EPA 2004), modified to reflect the hydrogeologic conditions in the area surrounding Barton Springs. New regionally-specific PRZM scenarios representing both agricultural and non-agricultural use sites were developed for the Barton Springs assessments, following the standard methodology for scenario development (U.S. EPA 2005). Using standard methods, runoff estimates predicted by the PRZM model are linked to the Exposure Analysis Modeling System (EXAMS), simulating the runoff entering a natural water body. For most ecological risk assessments, EFED uses a standard water body of fixed volume and geometry in EXAMS. EXAMS incorporates the processes of degradation and sorption expected to occur in ponds, canals, and first and second order streams. The standard water body is static (no outflow). Concentrations in larger water bodies are expected to be lower, thus the standard water body generally provides a conservative estimate of concentrations to which aquatic organisms may be exposed.

Because of the potentially rapid transit of the applied pesticide to Barton Springs via a ground water pathway, EFED opted to modify the standard methods, and calculate an estimated spring concentration rather than using the standard pond. A detailed explanation of the methodology is included in Appendix D.

3.2.1 *Background*

The Barton Springs salamander resides in a geographically limited area defined by a set of spring-fed pools in the outskirts of the city of Austin. All of the springs are fed via subsurface flow originating in fractured limestone (karst) of the Edwards Aquifer, which extends to the south-southwest away from the pool system. This area is known as the Barton Springs Segment of the Edwards Aquifer (BSSEA). The BSSEA includes four distinct hydrogeologic zones. From west to east, these are the Contributing Zone, the Recharge Zone, the Transition Zone, and the Artesian Zone.

Based on existing studies, surface water from the Contributing Zone and the Recharge Zone are most likely to contribute directly to the Barton Springs system (Slade *et al.,* 1985, Hauwert *et al.,* 2004). Ground water supplying the springs is derived from a combination of perennial ground water flow and recharge that originates from both infiltration of rainfall and downwelling from surface streams. Therefore, the exposure assessment focuses on the subsurface pathway delivering ground water to the springs.

An extensive summary of how ground water in the BSSEA system travels is provided in the ecological risk assessment for atrazine (EPA 2006b). This information is derived from a number of studies conducted by the U.S. FWS, the U.S.GS, and the City of Austin, and is considered best available data (Slade et al., 1986, Hauwert et al., 2004, USFWS 2005).

3.2.2 Exposure Modeling

The exposure modeling for the Barton Springs salamander takes the edge-of-field concentration estimate produced by PRZM for the standard field (converted to units of μg/L) and modifies it only by adjusting for the fraction of land use in the area contributing water to the springs that is classified as meadow (5%) (Equation 1). In essence, the standard field is presumed to be equivalent to the watershed, because as the field is increased in size, the runoff volume will increase proportionally. The "slug" of water leaving the field is assumed to arrive instantaneously in the springs. The salamander and its supporting ecosystem are presumed to be exposed to this concentration.[8]

Equation 1

PRZM Runoff Concentration(μg/L) * Meadow Land Use Fraction = EEC (μg/L)

The calculation for the estimated spring exposure estimate includes several conservative assumptions. It assumes: 1) metolachlor is applied simultaneously to all potential use sites (equivalent to 5% of the total land area, based on land use), 2) all the water arriving at the springs is derived from the runoff event, with no dilution either in the ground water system or in the spring pools, and 3) no degradation or sorption of the pesticide occurs during transit. Two other assumptions are inherent in the calculation as well, although the directional bias of these assumptions, if any, is unknown. These assumptions are 1) rainfall is distributed evenly across the area supplying water to the springs (comprised of the Contributing Zone, the Recharge Zone, and possibly the Transition Zone of the BSSEA), and 2) average runoff from all other land uses is approximately the same as runoff from the meadow scenario. EFED anticipates actual conditions are likely to result in lower metolachlor concentrations in the springs than estimated.

[8] Detailed calculations included in Appendix D

3.2.3 Label Application Rates and Intervals

The analysis of agricultural uses in the Barton Springs action area conducted by BEAD (Kaul and Carter 2005, D322226), and the additional analysis done in support of scenario development (SRC 2006) showed the only registered use site for metolachlor that may occur in the action area is application to sorghum. Sorghum grown in the BSSEA was identified by local agricultural experts as being for hay or silage. Land use areas where sorghum might be grown as those identified as meadow (Figure 6), which comprise 5% of the land use of the action area. EFED used the PRZM meadow scenario developed for Barton Springs, which more accurately reflects the agronomic practices used for hay than a standard small grain crop scenario might.

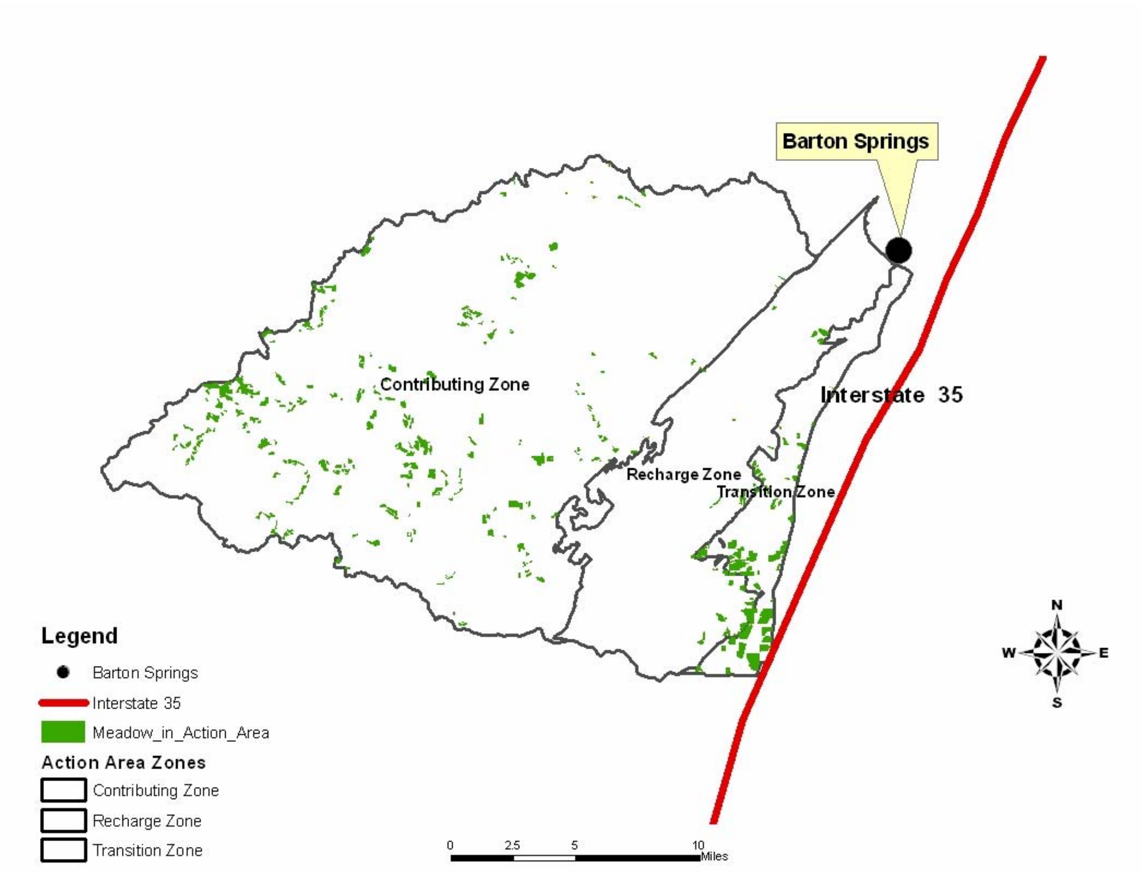

Figure 6 Locations of Meadow Land Use in Action Area

The initial label analysis conducted by BEAD (Kaul and Carter 2005, D322226) determined a maximum label rate for use of metolachlor on sorghum, as well as methods of application. Application methods allowed by existing labels include aerial, ground spray, chemigation, and soil incorporation. However, this analysis provided little insight into the timing or frequency of applications, which are not specified on the label. A more refined analysis (Kaul *et al*., 2006, D322226, D322266, and D322267) provided information regarding timing of application and average application rates, which are slightly lower than maximum label rates.[9] Maximum application rates were used in modeling. Generally, metolachlor appears to be applied in the spring, during the period prior to planting until crop emergence. The refined analysis noted all applications were liquid formulations, and frequency of application was once a year. No metolachlor use was reported in Texas between 2002 and 2005.

Timing of application can be an important factor in determining aquatic EECs, as the amount of rainfall in the period immediately following pesticide application will affect concentrations of pesticide in the runoff. In order to determine the most likely dates of sorghum planting and metolachlor application, EFED accessed several of the Texas state agricultural extension websites[10]. While there was extensive information available for planting dates of grain sorghum, the commodity calendar (http://agnews/tamu.edu/comcal/commodity) noted that forages and hays were planted at various times in all six agricultural regions of Texas. The three counties (Blanco, Hays, and Travis) in the BSSEA are located near the junction of the Central Texas and East Texas "planting/harvest" areas designated in the commodity calendar. Grain sorghum is not listed as a commodity grown in Central Texas. Planting dates for modeling were selected based on emergence dates included in the scenario documentation. Table 5 shows application rates, methods, and specific dates used in the exposure assessment.

Table 5 Label Application Rates

Application Rate (lb ai/A)	Application Timing	Application Date	Method
1.67	Prior to planting	February 15	Ground
1.67	At planting	February 25	Ground
1.67	Before crop emergence	March 1	Ground
1.67	Prior to planting	February 15	Aerial
1.67	At planting	February 25	Aerial
1.67	Before crop emergence	March 1	Aerial

9 The apparent difference between label rates and average application rates may be an artifact of the calculation process, which assumes that total pounds of active ingredient applied is distributed evenly across the total number of acres treated.

10 http://lubbock/tamu/edu/sorghum and http://agnews/tamu.edu/comcal/commodity

3.2.7 Exposure Modeling Input and Output

Table 6 shows input parameters for PRZM modeling, based on acceptable environmental fate data from guideline studies.

Table 6 Input Parameters for PRZM Modeling

Parameter	Value	Comments	Source
Application Rate (kg a.i./ha)[1]- ground or aerial spray	1.87		label
Molecular Weight (grams/mole)	283.8		
Solubility (mg/L)	4800	10X reported value	product chemistry
Vapor Pressure (torr)	$2.8E^{-5}$		
Henry's Constant (atm m^3/mol)	$3.75E^{-5}$		
K_d (L/kg)	181	Average K_{oc}[2]	MRID00078291 MRID43928935 MRID40430203 MRID40476404 MRID43928937 MRID40495602 MRID40495603 MRID40495604
Aerobic Soil Metabolism Half-life (days)	48.9	Estimated upper[3] 90th percentile	MRID41309801 MRID43928936 MRID45499606
Aerobic Aquatic Metabolism Half-life (days)	141	Based on 3X single aerobic aquatic metabolism linear first order half-life	MRID41185701
Anaerobic Aquatic Metabolism Half-life (days)	234	Based on 2X single anaerobic aquatic metabolism linear first order half-life	MRID41185701
Photodegradation in Water (days)	70		MRID40430202
Hydrolysis Half-life (days)	Stable		MRID40430201
Spray Drift Fraction	5% 1%	Aerial Ground	Default value

[1] - Application rate given in input units for PRZM-EXAMS. Conversion is kg/ha x 1.12 = lb/A
[2] – Sorption of metolachlor to soil is correlated with percent organic carbon, thus K_{oc} is a valid model for this compound. Average K_{oc} using values 118.5, 303.0, 151.4, 241.4, 66.8, 21.6, 110.4, 74.4, 175.0, 333.3, 230.0, 244.7, 226.3, 367.2, 176.5, 120.7, 111.1 as per "*Guidance for Chemistry and Management Practice Input Parameters for Use in Modeling the Environmental Fate and Transport of Pesticides"* dated February 28, 2002.
[3] - Upper 90th Percentile based on acceptable aerobic metabolism half-lives of 66, 37.8, 37.8, 14.9, 13.9, and 50.3 days.

Table 7 shows the estimated concentrations of metolachlor based on various application times and methods. As bounding estimates, the highest EECs and lowest EECs (denoted as [1] and [2], respectively) were used to calculate risk quotients (RQs). Peak concentrations ranged from 11.8 μg/L (ground spray, before crop emergence) to18.0 μg/L (aerial spray, prior to plant). At 21-days post application, concentrations ranged from 0.7 μg/L (ground spray, before crop emergence) to 1.0 μg/L (aerial spray, prior to plant). The 60-day post application concentrations ranged from 0.3 μg/L (ground spray, before crop emergence) to 0.4 μg/L (aerial spray, prior to plant).

Table 7 Estimated Concentrations for Metolachlor (@ 1.67 lbs ai/A) Based on a Texas Meadow Scenario

Application Technique	Application Timing	Peak	21 days	60 days
		ug/L		
Ground Spray	Prior to planting	17.228	0.968	0.394
Ground Spray	At Plant	14.655	0.729	0.342
Ground Spray	Before Crop Emergence	11.802[2]	0.657[2]	0.263[2]
Aerial Spray	Prior to planting	17.952[1]	1.010[1]	0.411[1]
Aerial Spray	At Plant	15.269	0.760	0.357
Aerial Spray	Before Crop Emergence	12.300	0.684	0.274

[1] Used as upper bound estimate for development of risk quotients (RQs)
[2] Used as lower bound estimate for development of risk quotients (RQs)

Although a complete fate data set was not available for the degradates metolachlor ESA and metolachlor OA, EFED used the PRZM model (Tier II) to estimated edge-of-field concentrations.

Limited data were available for modeling the degradates metolachlor ESA and metolachlor OA. These data included the soil adsorption/desorption studies for both ESA (MRID 44931722) and OA (MRID 40494605), as well as the conversion efficiency of metolachlor to the two degradates (MRIDs 43928936, 41309801). Application rates for the two degradates were determined by multiplying the maximum metolachlor application rate (1.87 lb ai/A) by the fraction of the relevant degradate. Half-lives for the two compounds were estimated using the decline portion of the formation and decline data contained in the Comparative Aerobic Soil Metabolism Study (MRID 43928936). For other parameters where data were not available, the compounds were conservatively assumed to be stable. PRZM input parameters are shown below.

Table 8 PRZM/EXAMS Parameters for Metolachlor ESA and Metolachlor OA

Parameter	Value	Comments	Source
Application Rate ESA (kg a.i./ha)	0.26	1.87 kg ai/ha * 1.16[1]*0.12	MRID43928936
Application Rate OA (kg a.i./ha)	0.52	1.87 kg ai/ha * 0.98[2]* 0.28	MRID41309801
Molecular Weight ESA (g/mole)	329.7		
Molecular Weight OA (g/mole)	279.4		
K_d ESA (L/kg)	0.041	Lowest non-sandy soil, Maryland clay	MRID44931722
K_d OA (L/kg)	0.079		MRID40494605
Solubility (mg/L)	4800	10X reported value of parent	product chemistry
Aerobic Soil Metabolism Half-life ESA (days)	162.5	Based on decline portion of formation and decline data	MRID4392836
Aerobic Soil Metabolism Half-life OA (days)	127.5		
Aerobic Aquatic Metabolism Half-life (days)	0	Assumed stable	
Anaerobic Aquatic Metabolism Half-life(days)	0		
Photodegradation in Water (days)	0		MRID40430202
Hydrolysis Half-life (days)	0		MRID40430201

[1]Molecular weight correction factor= MW ESA (329.7 g/mol)/ MW Metolachlor (283.8 g/mol)= 1.16
[2]Molecular weight correction factor= MW OA (279.4 g/mol)/ MW Metolachlor (283.8 g/mol) = 0.98

EECs for degradates are shown in Table 9. Peak concentrations for ESA ranged from 2.6 μg/L to 5.2 μg/L. For OA, the peak concentrations were slightly higher, ranging from 5.9 μg/L to 9.8 μg/L. The 21-day concentrations for ESA and OA, respectively, were 0.1-0.3 μg/L and 0.3-0.5 μg/L. At 60 days, the concentrations were estimated to range from 0.05 μg/L to 0.09 μg/L for ESA, and from 0.1 μg/L to 0.2 μg/L for OA.

Table 9 PRZM Estimated Estimated Spring Concentrations of Metolachlor ESA and Metolachlor OA

Application Timing	Peak		21 days		60 days	
	ug/L					
	ESA	OA	ESA	OA	ESA	OA
Prior to planting	4.368	9.754[1]	0.230	0.510[1]	0.080	0.178[1]
At Plant	5.251[1]	7.812	0.250[1]	0.372	0.089[1]	0.133
Before Crop Emergence	2.600[2]	5.983[2]	0.129[2]	0.298[2]	0.045[2]	0.105[2]

[1] Used as upper bound estimate for development of risk quotients (RQs)
[2] Used as lower bound estimate for development of risk quotients (RQs)

4.0 Effects Assessment

Acute toxicity data for metolachlor used to evaluate the assessment endpoints is presented in Table 10. EFED uses the most sensitive species in each evaluation category to assess risk. The complete set of toxicity data available to EFED at the time of the assessment is contained in Appendix B. The data set consists of toxicity data from acceptable guideline tests submitted to the Agency by the registrant and open literature toxicity data that meets established acceptability criteria ("ECOTOX data"). The complete data set includes values for both racemic metolachlor (PC108801) and S-metolachlor (PC108800). No open literature data were located for either metolachlor-ESA or metolachlor OA, thus this portion of the toxicity data only includes registrant-submitted guideline studies.

Metolachlor is slightly toxic to moderately toxic to fish (LC_{50}s 3.2-15.0 mg/L, Appendix B, Table 1 and Table 6) on an acute basis. Some amphibian data (Appendix B, Table 6) was located in ECOTOX. Toxicity data for two species, the African clawed frog (*Xenopus laevis*, LC_{50} 13.6 mg/L) and American bullfrog (*Rana catesbeiana*, EC_{50} 17.4 mg/L) indicated that mortality effects for amphibians occur in concentrations similar to lethal endpoints for fish, which serve as a surrogate for aquatic phase amphibians. Species sensitivity distributions for amphibians are not well understood at this point, thus EFED opted to use the more protective toxicity value from the fish data to calculate RQs. Metolachlor-ESA is slightly toxic to fish (LC_{50} 48 mg/L) and metolachlor-OA is practically non-toxic to fish (LC_{50} >93.1 mg/L). No amphibian data were located for the degradates. Sub-lethal effects noted in tests include lethargy and loss of equilibrium, occurring at concentrations of $\geq$ 3.3 mg/L. The NOAEC in chronic tests (fathead minnow) is 1 mg/L.

Metolachlor is slightly toxic to moderately toxic to freshwater invertebrates (EC_{50}s 3.8-26.0 mg/L, Appendix B, Table 1 and Table 6).The lowest chronic toxicity value for tests that evaluated decreases in survival, reproduction and growth was for the water flea (*Daphnia magna*, NOAEC 3.2 mg/L). One study from open literature noted sublethal effects (behavioral modifications) in rusty crayfish *(Oronectes rusticus)* at metolachlor concentrations of 0.025 mg/L(Appendix B, Table 6). Metolachlor-ESA is practically non-toxic and metolachlor-OA is slightly toxic to the water flea (*Daphnia magna).* No chronic toxicity data were located for the degradates.

Based on registrant-submitted data, green algae are the most sensitive aquatic plants (EC_{50} 0.008 mg/L, NOAEC 0.002 mg/L), and, as expected for an herbicide, plants are several orders of magnitude more sensitive than the aquatic animals. Toxicity values for various genera of aquatic plants ranged from 0.008 mg/L (green algae, S-metolachlor) to 1.2 mg/L (bluegreen algae, racemic metolachlor). Duckweed (*Lemna gibba*, EC_{50} 0.048 mg/L) which is the surrogate for aquatic vascular plants, is less sensitive to the effects of metolachlor than the green alga, but more sensitive than any of the other non-vascular aquatic plants. For three genera, toxicity data were available for both racemic metolachlor and S-metolachlor. Based on these data, green algae and duckweed are slightly more sensitive to S-metolachlor, and saltwater diatoms are less sensitive. The more sensitive S-metolachlor data are used in this assessment. Green algae and duckweed are much less sensitive to both metolachlor degradates (EC_{50}s >40mg/L).

Table 10 Aquatic Toxicity Profile for Metolachlor

Assessment Endpoint	Surrogate Species	Toxicity Value Used	Source Citation	Comments
Direct Effects				
Acute Toxicity to Salamander	Bluegill sunfish	LC_{50} = 3.2 mg/L 95% CI = 2.8-4.6 mg/L Slope = 14.8	MRID 43928910	Sub-lethal effects: loss of equilibrium (3.3 ppm)
Chronic Toxicity to Salamander	Fathead minnow	NOAEC = 1 mg/L LOAEC = 2.2 mg/L	MRID 43044602	Increase in mortality noted at ≥5 ppm Hatch rate affected at 8.6 ppm
Indirect Effects (Prey Reduction))				
Acute Toxicity to Prey	Water flea	EC_{50} = 1.1 mg/L	ECOTOX 67777	Immobilization (*i.e.* mortality) was endpoint measured
Chronic Toxicity to Prey	Water flea	NOAEC = 0.001 mg/L LOAEC = 0.01 mg/L	ECOTOX 83887	Most sensitive endpoint number of young per female
Indirect Effects (Habitat Modification)				
Acute Toxicity to Plants (non-vascular)	Green algae	LC_{50} = 0.008 mg/L 95% CI =0.003-0.025 mg/L Slope = 3 NOAEC = 0.0015 mg/L	MRID 43928929	None
Acute Toxicity to Plants (vascular)	Duckweed	LC_{50} = 0.021 mg/L 95% CI =0.019-0.023 mg/L NOAEC=0.0076 mg/L	MRID 43928931	None

Table 11 Aquatic Toxicity Profile for Degradate Metolachlor OA

Assessment Endpoint	Surrogate Species	Toxicity Value Used	Source Citation	Comments
Direct Effects				
Acute Toxicity to Salamander	Crucian carp	LC_{50} = >93.1 mg/L NOAEC = >96.3 mg/L	MRID 44929502	None
Chronic Toxicity to Salamander	No data available			
Indirect Effects (Prey Reduction))				
Acute Toxicity to Prey	Water flea	LC_{50} = 15.4 mg/L 95%CI=13.0-18.4 mg/L Slope = 6.1	MRID 44929503	None
Chronic Toxicity to Prey	No data available			
Indirect Effects (Habitat Modification)				
Acute Toxicity to Plants (non-vascular)	Green algae	LC_{50} = 57.1 mg/L NOAEC = 29.3 mg/L	MRID 4492515	None
Acute Toxicity to Plants (vascular)	Duckweed	LC_{50} = >95.1 mg/L NOAEC = 95.4	MRID 4429514	None

Table 12 Aquatic Toxicity Profile for Degradate Metolachlor ESA

Assessment Endpoint	Surrogate Species	Toxicity Value Used	Source Citation	Comments
Direct Effects				
Acute Toxicity to Salamander	Rainbow trout	LC_{50} = 48 mg/L 95% CI = 36-64 mg/L NOAEC = 36 mg/L	MRID 449931702	Sub-lethal effects at ≥58 ppm : loss of equilibrium, erratic swimming, pigmentation changes
Chronic Toxicity to Salamander	No data available			
Indirect Effects (Prey Reduction))				
Acute Toxicity to Prey	Water flea	LC_{50} = >108 mg/L NOAEC = 108 mg/L	MRID 44931703	108 ppm highest concentration tested
Chronic Toxicity to Prey	No data available			
Indirect Effects (Habitat Modification)				
Acute Toxicity to Plants (non-vascular)	Green algae	LC_{50} = >99.45 mg/L NOAEC = 99.45 mg/L	MRID 44931720	None
Acute Toxicity to Plants (vascular)	Duckweed	LC_{50} = >95.1 mg/L NOAEC = 95.4	MRID 44931719	None

4.1 Summary of Aquatic Ecotoxicity Studies

Information used to develop the toxicity profile for metolachlor included registrant-submitted guideline studies for both racemic metolachlor and S-metolachlor, and open literature studies that met the criteria for inclusion into ECOTOX. Open literature studies generally do not indicate whether the active ingredient tested was racemic metolachlor or S-metolachlor. The lowest values for various taxon, used to derive RQs, are reported in Table 10. Data for the degradates are reported in Table 11 and Table 12. In all cases the lowest available endpoint (based on LC_{50} for acute tests, and NOAEC for chronic tests) was used in the calculation.

4.1.1 Toxicity to Freshwater Fish

4.1.1.1 Acute Exposure (Mortality) Studies

A number of guideline studies evaluating the acute effects of metolachlor on freshwater fish were available. LC_{50}s for fish ranged from 3.2 mg/L to 15.0 mg/L, classifying metolachlor as moderately to slightly toxic to fish. Sub-lethal effects noted in several studies included loss of equilibrium and lethargy. Generally, sub-lethal effects occurred at the same concentrations as mortality. A number of different species were considered. No obvious pattern related to species sensitivity distribution was noted. (*e.g.,* warm water fish being more or less sensitive than coldwater fish.) Data from ECOTOX studies (ECOTOX #6797) gave the LC_{50} as 8.0-8.4 mg/L.

An acute toxicity study assessing the effects of metolachlor-ESA (MRID 44931702) on rainbow trout *(Onchorynchus mykiss)* showed the degradate to be less toxic than the parent. The LC_{50} was 48 mg/L, classifying metolachlor ESA as slightly toxic to fish. In concentrations where mortality occurred, sub-lethal effects noted included erratic swimming, loss of equilibrium, and pigmentation changes.

Acute toxicity studies were available for the effects metolachlor-OA on two fish species, crucian carp *(Carassius carassius* MRID 44929501*)*, and rainbow trout (*Onchorynchus mykis*s, MRID 44929502). The degradate is practically non-toxic to fish on an acute basis with LC_{50}s of >93.1 mg/L and >96.3 mg/L, respectively.

4.1.1.2 Chronic Exposure (Growth/Reproduction) Studies

The only chronic study available for freshwater fish was a registrant-submitted study on fathead minnow (*Pimephales promela*s). The NOAEC for the most sensitive endpoint, dry weight of the larval fish, was 0.030 mg/L. The LOAEC was 0.056 mg/L.

4.1.2 Toxicity to Aquatic Phase Amphibians

No guidelines currently exist for amphibian toxicity studies. However, several studies evaluating the acute and chronic effects of metolachlor on two species of frogs met the criteria for inclusion into ECOTOX. Neither of these studies met acceptability criteria for inclusion into the assessment as a quantitative endpoint. Endpoints derived from these studies occurred at higher concentrations than the effects reported for the guideline fish studies, which are typically used as a surrogate for amphibians. Because of these facts, EFED has elected to use the more protective fish-derived toxicity values in this assessment.

4.1.2.1 Acute Exposure (Mortality) Studies

Two acute toxicity studies for amphibians were reported in ECOTOX, one for the African clawed frog (*Xenopus laevis*, LC_{50} 13.6 mg/L) and one for the American bullfrog (*Rana catesbeiana*, EC_{50} 17.4 mg/L). These values are higher than almost all of the LC_{50}s reported for fish. Based on these data, metolachlor would be classified as slightly toxic to amphibians.

For the bullfrog, the test consisted of exposing tadpoles to the metolachlor-containing formulation DUAL-960E for 24 hours under static conditions (ECOTOX #20274). The study contained no mention of analyzing the solution for active ingredient, thus EFED assumes the reported concentrations are nominal. Sublethal effects reported in this study include cellular damage (LOAEL 0.272 mg/L). This study was considered for qualitative use only in the assessment because a formulation was tested rather than the technical active ingredient.

The study on the African clawed frog (ECOTOX # 66376) exposed embryos from wild-collected frogs to static concentrations of metolachlor (reported purity 99%). The reported 96-hour LC_{50} for metolachlor was 13.6 mg/L. The study also determined 96-hour LC_{50}s for two degradation products of metolachlor (2,6-diethylaniline and 2-ethyl-6-methylaniline). These LC_{50}s were 19.4 mg/L and 68.8 mg/L, respectively. Based on guideline fate studies, degradates evaluated in this study are not considered "major" degradates of metolachlor, and are not addresssed in this assessment. Sublethal effects in embryos exposed to metolachlor included edema, gut malformations, axial flexures, and eye abnormalities. Similar effects were noted for the degradates, although to a lesser extent. This study was considered for qualitative use only because the parent test frogs were wild-caught, and previous exposure to other chemicals (which could modify sensitivity) cannot be ruled out.

4.1.3 Toxicity to Freshwater Invertebrates

4.1.3.1 Acute Exposure (Mortality) Studies

Registrant-submitted toxicity tests show metolachlor (MRID 00015546) and s-metolachlor (MRID 43928912) to be slightly toxic to daphnids on an acute basis. LC_{50}s for *Daphnia magna* ranged from 25-26 mg/L. NOAECs from these studies were 5.6 mg/L and 4.8 mg/L, respectively. Sublethal effects included lethargy.

Several open literature studies were available in ECOTOX for aquatic invertebrates. While some produced EC_{50}s in the same range (~25 mg/L) as the guideline tests, others were nearly an order of magnitude lower, in the 1.1-4.4 mg/L range.

The lowest endpoint from the open literature studies, for the water flea (*Ceriodaphnia dubia,* ECOTOX#6777) was used to calculate RQs for this assessment. Test methods were based on USEPA (1991). The test endpoint was immobilization, examined under dark field illumination at 6.5X magnification. In laboratory water the 48-hr EC_{50} (95% CI) was 1.1 mg/L (0.9-1.4 mg/L). These results are consistent with other toxicology data on metolachlor, and with species sensitivity distribution of aquatic invertebrates.

In a midge fly larvae (*Chironomus plumosus,* ECOTOX# 6797) study, both technical metolachlor (95.4% purity) and an emulsifiable concentrate (87% a.i.) were used in 48-hour static tests. The LC_{50}s for the tests were 3.8 mg/L (technical) and 4.4 mg/L (concentrate).

Guideline studies on *Daphnia magna* were submitted for both major degradates. The LC_{50} for metolachlor-OA is 15.4 mg/L (MRID 44929503), classifying it as slightly toxic to aquatic invertebrates. For the metolachlor-ESA, the LC_{50} was >108 mg/L, (MRID 44931703), classifying it as practically non-toxic to aquatic invertebrates.

4.1.3.2 Chronic Exposure (Growth/Reproduction) Studies

The registrant submitted a full life cycle study assessing the effects of metolachlor on *Daphnia magna* (MRID 43802601). Measured concentrations were highly variable throughout the study, thus the lowest measured concentrations were used to derive conservative endpoints. Based on growth and reproduction, the NOAEC and LOAEC were 3.2 and 6.9 mg/L, respectively.

A study comparing the effects of racemic metolachlor and S-metolachlor was located (ECOTOX #83887). Authors note the test design is in accordance with OECD (1995) and ISO (1996) guidance for toxicity tests using *Daphnia magna*. Parameters measured in chronic test included length, longevity, days to first brood, broods per female, number of young per female. Concentration of pesticide in stock solution was determined analytically (HPLC), with 95-97% of original concentration remaining after one week. Stock solutions were renewed weekly during the test to minimize degradation of the compound. Authors do not describe analytical measurements of test solutions, thus concentrations are considered to be nominal. The most sensitive parameter was the number of young per female, which was significantly different at 0.01 mg/L for racemic metolochlor, and 0.5 mg/L for S-metolachlor. Other measured parameters were not significantly different until concentrations reached 1mg/L. For 3 out of 5 parameters measured, racemic metolachlor was toxic to daphnids at a lower concentration than S-metolachlor. For one parameter (length), effects were significant at the same concentration. Days to first brood was not affected at concentrations tested for either chemical. Based on this study the chronic endpoints are:

Racemic metolachlor	NOAEC 0.001 mg/L	LOAEC 0.01 mg/L
S-metolachlor	NOAEC 0.1 mg/L	LOAEC 0.5 mg/L

4.1.3.3 Sublethal Effects

One study located in the open literature evaluated effects of technical metolachlor on the behavior of rusty crayfish (*Oronectes rusticus*, ECOTOX #68515). Crayfish were collected from the wild and tested for their ability to respond appropriately to odor cues following exposure to metolachlor. Both a positive (food odor) and negative (predator odor) cue were tested. Measurements included length of time to locate the odor source, percent success in locating the odor source, and time spent motionless. Concentrations of metolachlor tested ranged from 25-75 ppb, and included both a negative control and a solvent control. At a concentration of 25 ppb, the crayfish had less success in finding the food source, took longer to find the food source, and exhibited modifications in alarm response. There appeared to be a dose-response relationship. Based on this study, the behavioral NOAEC is <25 ppb and the LOAEC is 25 ppb. These endpoints are only used qualitatively in the assessment because the effects cannot be quantitatively linked to the assessment endpoints of survival, growth, and reproduction.

4.1.4 Toxicity to Aquatic Plants

EFED evaluated both registrant submitted studies and open literature studies for aquatic plants. Overall, based on a review of the data, the endpoints and test durations used by independent evaluators are similar to those in the guideline studies. Guideline studies provided more sensitive endpoints, and these were used in the assessment.

The registrant submitted aquatic plant studies for racemic metolachlor, s-metolachlor and for the two major degradates, metolachlor-ESA and metolachlor-OXA. For the racemic metolachlor testm all five standard aquatic plant species wer tested. EC_{50} values ranged from 0.010 mg/L (green alga) to 1.2 mg/L (blue-green alga). NOAEC s ranged from 0.0007 mg/L (green alga) to 0.063 (blue-green alga). For s-metolachlor, data were submitted for the three aquatic plants most sensitive to metolachlor (green algae, duckweed, marine diatom). S-metolachlor EC_{50}s ranged from 0.008 mg/L (green alga) to 0.11 mg/L. NOAEC values ranged from 0.0015 mg/L (green alga) to 0.021 mg/L (marine diatom). Each of the two major degradates was tested with both a non-vascular (green alga) and a vascular (duckweed) plant. Both degradates are less toxic to aquatic plants than the parent compounds. Duckweed is the more sensitive of the two plants test to metolachlor–ESA, with an EC_{50} of 43 mg/L and a NOAEC of 4 mg/L. Green alga is the more sensitive to OXA, with an EC_{50} of 57 mg/L and a NOAEC of 29 mg/L.

4.2 Use of Probit Slope Response Relationship

Generally, available toxicity data provides and LC_{50} or an EC_{50}, (the concentration at which 50% of the test populatin exhibits the designated endpoint, usually mortality). Because the Endangered Species Act (ESA) requires determination of potential effects at an individual level, this information must be extrapolated from existing data. The Agency uses the probit dose response relationship as a tool for deriving the probability of effects on a single individual (U.S. EPA, 2004). The individual effects probability associated with the acute RQ is based on the mean estimate of the probit dose response slope and an assumption of that probit model is appropriate for the data set. In some cases, probit is not the appropriate model for the data, and EFED has low confidence in extrapolations from these types of data sets. In addition to a single effects probability estimate based on the mean, upper and lower estimates of the effects probability are also provided to account for variance in the slope, if available. The upper and lower bounds of the effects probability are based on available information on the 95% confidence interval of the slope. Individual effect probabilities are calculated based on an Excel spreadsheet tool IECV1.1 (Individual Effect Chance Model Version 1.1) developed by the U.S. EPA, OPP, Environmental Fate and Effects Division (June 22, 2004). Probability of individual effects for the various assessment endpoints is provided below in Table 13.

Table 13 Probability of Individual Effects

Assessment Endpoint	Surrogate Species	LC_{50}(mg/L) and Slope	Fits Probit	Chance of Individual Effect
Direct Effects				
Acute Toxicity to Salamander	Bluegill sunfish	3.2 and 2.1 (slope lower bound) 3.2 and 14.8 (slope) 3.2 and 27.5 (slope upper bound)	Yes	1 in 318 <1 in 10^{16} <1 in 10^{16}
Chronic Toxicity to Salamander	Fathead minnow	Evaluated based on no effects level, chance of effects evaluation not required		
Indirect Effects (Prey Reduction)				
Acute Toxicity to Prey	Water flea	1.1 and 4.5 (default slope)	Unknown (raw data not available to calculate)	1 in $4.2x10^{8}$
Chronic Toxicity to Prey	Water flea	Evaluated based on no effects level, chance of effects evaluation not required		
Indirect Effects (Habitat Modification)				
Acute Toxicity to Plants (vascular)	Duckweed	Evaluated based on no effects level, chance of effects evaluation not required		
Acute Toxicity to Plants (non-vascular)	Green algae	Evaluated based on no effects level, chance of effects evaluation not required		

4.3 Incident Database Review

The incident database contains a total of 171 reports for metolachlor. Of the reports, 150 are of plant damage mostly to agricultural crops under registered use conditions. The most commonly reported crop damage was to corn, peanuts, and soybeans. There was one reported bird kill that was rated as unlikely to be associated with the metolachlor use. There are 19 reported incidents of effects on aquatic animals, primarily fish. Generally, these occurred under registered use conditions, and were rated as possibly or unlikely to be associated with the application of metolachlor. One incident, a fish kill in Minnesota, has a certainty rating of highly probable, but was also listed as accidental misuse.

Incidents are reported separately for S-metolachlor, but the number and type of reports are similar. There were total of 117 reported incidents for S-metolachlor. Of these, only two reports are for organisms other than plants. In one case, there is a report of three birds dying as a result of S-metolachlor use. The certainty of this incident was unrated, and legality designated as unknown. The second case was a reported fish kill, of an unspecified magnitude. The legality of the use was designated unknown, and the incident was designated unlikely to be the result of the pesticide use. The remainder was damage to agricultural crops. Based on the data, it appears that most of the reports are undesired effects on the at the treatment site, when applied in accordance with registered use. The most commonly reported damaged crops were corn, cotton, and soybean. The certainty was generally rated as possible.

5.0 Risk Characterization

5.1 Risk Estimation

To estimate risk, EFED calculates risk quotients (RQs; estimated exposure/toxicity value), and compares them the pre-established levels of concern (LOCs). Exceedence of an LOC indicates potential risk to the class of organisms for which the LOC is exceeded (direct effects) and for organisms which may depend on the class of organisms potentially at risk (indirect effects). In this assessment, EFED has calculated risk quotients for metolachlor (Table 14) and the two major degradates (OA, Table 15 and ESA, Table 16) for each assessment endpoint. Risk quotients are calculated for two types of exposure: background concentrations, and EECs in the spring based on PRZM modeling. For the modeled concentrations, two RQs are given as bounding estimates for the range of application methods and dates modeled in PRZM.

No RQs for background concentrations of metolachlor (based on monitoring data) exceed any LOCs for any taxa.

For metolachlor, no LOCs are exceeded for acute or chronic effects directly on the salamander. RQs based on all estimates are <0.05. No LOCs are exceeded for the degradate metolachlor OA (Table 15) or the degradate metolachlor ESA (Table 16).

No acute LOCs are exceeded for aquatic invertebrates (potential prey reduction effects). The chronic RQ (1.01) for the highest modeled EEC in the springs exceeds the LOC, but the RQ (0.66) for the lowest modeled EEC does not. Aquatic invertebrates are a primary food source for the Barton Springs salamander.

RQs based on modeled upper and lower bound EECs, (2.2 and 1.5 respectively) exceed the acute risk LOC for green algae, the most sensitive non-vascular aquatic plant tested. The green alga, an aquatic non-vascular plant, represents the primary producers (plankton and periphyton) in the Barton Springs foodchain. Reduction of primary producers would constitute habitat modification, and would be considered an indirect effect on the Barton Springs salamander.

Table 14 Risk Quotients for Metolachlor

Assessment Endpoint	Surrogate Species	Concentration Estimate	RQ	LOC Exceedence[1]
Direct Effects				
Acute Toxicity to Salamander	Bluegill sunfish	Background Estimated spring (highest) Estimated spring (lowest)	<0.05 <0.05 <0.05	No No No
Chronic Toxicity to Salamander	Fathead minnow	Background Estimated spring (highest) Estimated spring (lowest)	<1.0 <1.0 <1.0	No No No
Indirect Effects (Prey Reduction))				
Acute Toxicity to Prey	Midge fly larvae	Background Estimated spring (highest) Estimated spring (lowest)	<0.05 <0.05 <0.05	No No No
Chronic Toxicity to Prey	Water flea	Background Estimated spring (highest) Estimated spring (lowest)	<1.0 1.01 <1.0	No *Yes* No
Indirect Effects (Habitat Modification)				
Acute Toxicity to Plants (vascular)	Duckweed	Background Estimated spring (highest) Estimated spring (lowest)	<1.0 <1.0 <1.0	No No No
Acute Toxicity to Plants (non-vascular)	Green algae	Background Estimated spring (highest) Estimated spring (lowest)	<1.0 2.15 1.48	No *Yes* *Yes*

[1] LOCs used in this assessment:
Aquatic animals acute risk endangered species 0.05
Aquatic animals chronic risk 1.0
Aquatic plants acute risk 1.0.

Table 15 Risk Quotients for Degradate Metolachlor OA

Assessment Endpoint	Surrogate Species	Concentration Estimate	RQ	LOC Exceedence
Direct Effects				
Acute Toxicity to Salamander	Bluegill sunfish	Background Estimated spring (highest) Estimated spring (lowest)	NC <0.05 <0.05	No No No
Chronic Toxicity to Salamander	Fathead minnow	Background Estimated spring (highest) Estimated spring (lowest)	NC <1.0 <1.0	No No No
Indirect Effects (Prey Reduction))				
Acute Toxicity to Prey	Water flea	Background Estimated spring (highest) Estimated spring (lowest)	NC <0.05 <0.05	No No No
Chronic Toxicity to Prey	No data available			
Indirect Effects (Habitat Modification)				
Acute Toxicity to Plants (vascular)	Duckweed	Background Estimated spring (highest) Estimated spring (lowest)	NC <1.0 <1.0	No No No
Acute Toxicity to Plants (non-vascular)	Green algae	Background Estimated spring (highest) Estimated spring (lowest)	NC <1.0 <1.0	No No No

NC Not calculated because there are no monitoring data for degradates
[1] LOCs used in this assessment:
Aquatic animals acute risk endangered species 0.05
Aquatic animals chronic risk 1.0
Aquatic plants acute risk 1.0.

Table 16 Risk Quotients for Degradate Metolachlor ESA

Assessment Endpoint	Surrogate Species	Concentration Estimate	RQ	LOC Exceedence
Direct Effects				
Acute Toxicity to Salamander	Bluegill sunfish	Background Estimated spring (highest) Estimated spring (lowest)	NC <0.05 <0.05	No No No
Chronic Toxicity to Salamander	Fathead minnow	Background Estimated spring (highest) Estimated spring (lowest)	NC <1.0 <1.0	No No No
Indirect Effects (Prey Reduction))				
Acute Toxicity to Prey	Water flea	Background Estimated spring (highest) Estimated spring (lowest)	NC <0.05 <0.05	No No No
Chronic Toxicity to Prey	No data available			
Indirect Effects (Habitat Modification)				
Acute Toxicity to Plants (vascular)	Duckweed	Background Estimated spring (highest) Estimated spring (lowest)	NC <1.0 <1.0	No No No
Acute Toxicity to Plants (non-vascular)	Green algae	Background Estimated spring (highest) Estimated spring (lowest)	NC <1.0 <1.0	No No No

NC Not calculated because there are no monitoring data for degradates
[1] LOCs used in this assessment:
Aquatic animals acute risk endangered species 0.05
Aquatic animals chronic risk 1.0
Aquatic plants acute risk 1.0.

5.2 *Risk Description*

5.2.1 Direct Effects

Based on the available toxicity information at the time of this assessment, potential uses of metolachlor in the Barton Springs salamander action area, and lack of LOC exceedences EFED does not anticipate any direct reduction in the survival, growth, or reproduction of the Barton Springs salamander when the pesticide is used in accordance with the approved label. At the lower bound of the 95% confidence interval of the probit slope, probability of mortality of an individual salamander is 1 in 318. Based on the mean estimate of the slope, probability of mortality of an individual salamander is <1 in 10^{16}. At the upper bound of the 95% confidence interval of the probit slope, probability of mortality of an individual salamander is <1 in 10^{16}.

Sublethal effects reported in literature include reduced response to olfactory stimulus in rusty crayfish (ECOTOX #68515). The reported LOAEC for this study was 0.025 mg/L metolachlor. This concentration was not exceeded by the highest peak EEC in the spring (0.017 mg/L). As the study did not determine a NOAEC, behavioral effects cannot be completely ruled out, but on the basis of existing data they do not appear likely.

5.2.2 Indirect Effects (Reduction in Prey Base)

Estimated concentrations in the springs exceed the most sensitive chronic risk endpoint (NOAEC 1 μg/L) for aquatic invertebrates, but are an order of magnitude less than the concentration at which effects were noted (LOAEC 10 μg/L). Of the six EECs (ground and aerial applications, three different application dates each), only the concentrations based on pre-plant applications equaled the NOAEC. Thus, if metolachlor was applied simultaneously to fields comprising 5% of the land area of the BSSEA, it could have a chronic effect on aquatic invertebrates if the effects endpoint is closer to the NOAEC than the LOAEC. Other studies containing chronic or sublethal endpoints for aquatic invertebrates report the lowest observed effects at concentrations ranging from 6.9 mg/L (*Daphnia magna*, MRID 43802601) to 25 μg/L (rusty crayfish, ECOTOX 68515).

No acute risk RQs exceed the endangered species LOC, thus EFED does not anticipate a reduction in prey base due to acute mortality. Based on the EC_{50} and a default slope for the probit analysis, the probability of mortality of an aquatic invertebrate due to metolachlor use is 1 in 4.2×10^8.

Chronic effects could occur for aquatic invertebrates exposed to metolachlor derived from applications originating in the BSSEA. However, based on the facts that 1) there are very limited pasture/meadow land uses in the BSSEA, 2) there was no reported use of metolachlor[11] in Texas in 2000-2005, and 3) the EEC is nearly an order of magnitude below the LOAEC, EFED anticipates the likelihood of chronic effects on aquatic invertebrates is low.

[11] Metolachlor is only registered for agricultural uses, which are generally well tracked and reported. The same generalization might not apply to pesticides registered for residential or homeowner use.

5.2.3 Indirect Effects (Habitat Degradation)

As part of the indirect effects analysis, reduction of both non-vascular plants and vascular plants in the Barton Springs system is considered. Non-vascular plants (plankton, periphyton, and some bryophytes) are primarily a food source for the salamander's prey items. Vascular plants serve as structure in the Barton Springs system, providing attachment points for periphyton, and refugia for both the salamander and its prey.

RQs based on the estimated spring concentration for the most sensitive non-vascular plant (green alga) exceed the LOC. In order to further characterize this potential risk, EFED calculated RQs for the upper and lower bounds of the 95% confidence interval for the green alga, and RQs for other types of freshwater non-vascular plants. RQs for green algae based on the lower bound of the confidence interval exceeded the LOC, but RQs based on the upper bound did not. RQs calculated for the freshwater diatom and for blue-green algae using the estimated spring concentration did not exceed the LOC. Based on this information, EFED concludes that if estimated spring EECs did occur in the springs, it could cause a shift in the algal assemblage in the spring as the populations of more sensitive species are reduced or removed. However, given several conservative estimates built into the exposure modeling, data regarding the usage of metolachlor in the BSSEA, and monitoring data, it appears unlikely those concentrations will occur.

Table 17 Comparison of Aquatic Non-Vascular Plant Assessment Endpoints and Estimated Spring EECs

Assessment Endpoint	Surrogate Species	Concentration Estimate	RQ	LOC Exceedence
Indirect Effects (Habitat Modification)				
Acute Toxicity to Plants (non-vascular) Based on LC_{50} for most sensitive species	Green algae	Estimated spring (highest) Estimated spring (lowest)	2.26 1.78	*Yes* *Yes*
Acute Toxicity to Plants (non-vascular) Based on 95% CI lower bound for most sensitive species	Green algae	Estimated spring (highest) Estimated spring (lowest)	6.03 4.74	*Yes* *Yes*
Acute Toxicity to Plants (non-vascular) Based on 95% CI upper bound for most sensitive species	Green algae	Estimated spring (highest) Estimated spring (lowest)	<1.0 <1.0	No No
Acute Toxicity to Plants (non-vascular) Based on LC_{50} for less sensitive species	FW diatom	Estimated spring (highest) Estimated spring (lowest)	<1.0 <1.0	No No
	Blue-green algae	Estimated spring (highest) Estimated spring (lowest)	<1.0 <1.0	No No

5.3 *Risk Conclusions*

After completing the analysis of the effects of metolachlor on the Federally listed endangered Barton Springs salamander *(Eurycea sosorum)* in accordance with methods delineated in the Overview document (USEPA 2004), EFED concludes that the use of metolachlor (PC 108801) may affect, but is not likely to adversely affect the Barton Springs salamander, based on indirect effects. Potential but not anticipated indirect effects include reduction of the prey base and/or reduction of primary productivity in the spring system. Rationale for each component assessed is provided in Table 18.

Table 18 Effects Determination for Metolachlor

Assessment Endpoint	Effects determination	Basis for Determination
	Direct Effects	
Survival, growth, and reproduction of Barton Springs salamander	No effect	No LOC exceedences for surrogate taxa (freshwater fish) representing Barton Springs salamander.
	Indirect Effects	
Reduction of prey (*i.e.,* freshwater invertebrates)	May affect Not likely to adversely affect (Discountable)	No acute LOC exceedences for freshwater invertebrates. Chronic LOC exceedence at highest peak modeled concentrations, but not any others. Effects noted in study used to establish assessment endpoint occurred at concentrations an order of magnitude higher than modeled concentrations. Modeled concentrations are 5 orders of magnitude higher than monitored concentrations.
Degradation of habitat and/or primary productivity (*i.e.,* aquatic plants)	May affect Not likely to adversely affect (Discountable)	LOC exceedences at modeled peak EEC for most sensitive freshwater plant species (green alga), but not for any other freshwater plant species for which data was available. No exceedences based on monitored concentrations.

6.0 Uncertainties

Risk assessment, by its very nature, is not exact, and requires the risk assessor to make assumptions regarding a number of parameters, to use data which may or may not accurately reflects the species of concern, and to use models which are a simplified representation of complex ecological processes. In this risk assessment, EFED has attempted to locate the best available data regarding such important parameters as the life history of the Barton Springs salamander, typical environmental conditions in the proximity of Barton Springs, toxicity of metolachlor, and uses of metolachlor in the action area. Frequently, such information are better expressed as ranges rather then points, and when this is the case, EFED has opted to make use of the end of range which would result in a conservative estimate of risk, in order to provide the benefit of doubt to the species. These uncertainties, and the directions in which they may bias the risk estimate, are described below.

6.1 Exposure Assessment Uncertainties

Overall, the uncertainties inherent in the exposure assessment tend to result in over-estimation of exposures. This is apparent when comparing modeling results with monitoring data. In particular, estimated peak exposures are generally 3-4 orders of magnitude above the highest detections in any of the four springs or surface waters in the Barton Springs area. In general, the monitoring data should be considered a lower bound on exposure, while modeling represents an upper bound. Factors influencing the over-estimation of exposure include the assumptions of no degradation, dilution, or mixing in the subsurface transport from Estimated spring to the Barton Springs complex. The modeling exercise conservatively assumes that the spring and the application site are adjacent. In reality, they are not, and there are likely to be environmental processes not accounted for that will reduce the predicted exposures.

6.1.1 Modeling Assumptions

The uncertainties incorporate in the exposure assessment cannot be quantitatively characterized. However, given the available data and EFED's policy to rely on conservative modeling assumptions, it is expected that the modeling results in an over-prediction in exposure. Qualitatively, conservative assumptions which may affect exposure include the following:

- The assessment assumes all applications have occurred concurrently on the same day at the exact same application rate.
- The assessment assumes all applications are at maximum label rate.

6.2.2 Impact of Vegetative Setbacks on Runoff

EFED does not currently have an effective tool to evaluate the impact of vegetative setbacks on runoff and pesticide loadings. The effectiveness of such setbacks is highly dependent on the condition of the vegetative strip. A well-established, healthy vegetative setback can be a very effective means of reducing runoff and erosion from agricultural fields and may substantially reduce loading to aquatic ecosystems. However, a setback that is narrow, of poor vegetative quality, or channelized is likely to be ineffective at reducing loadings. The presence and quality of setbacks are site-specific, and may vary widely, even within a small geographic area. EFED does not currently incorporate any "buffer reduction" in its exposure estimates. Until such time as quantitative methods to estimate the effect of vegetative setbacks of various conditions on pesticide loadings become available, EFED's aquatic exposure predictions are likely to overestimate exposure where healthy vegetative setbacks exist and may underestimate exposure where poorly developed, channelized or no setbacks exist.

6.2.3 PRZM Modeling inputs and Predicted Aquatic Concentrations

EFED currently typically uses the linked PRZM/EXAMS model which produces estimated aquatic concentrations based on site conditions and historical meteorological files (generally 30-year), although for this assessment, EXAMS has been decoupled, and other methods are used to estimate water concentrations. The "peak" pesticide concentration used in the assessment is probability-based, and is expected to be exceeded once within a ten-year period. PRZM is a process-based "simulation" model, which calculates what happens to a pesticide in a farmer's field on a day-to-day basis. It considers factors such as rainfall and plant transpiration of water, as well as how and when the pesticide is applied. The two major components are hydrology and chemical transport. Water movement in and off the field is simulated by the use of generalized soil parameters, including field capacity, wilting point, and saturation water content. Soils in each scenario are selected to represent high availability conditions for the pesticide. The chemical transport component simulates the method of pesticide application on the soil or on the plant foliage and the environmental processes acting on the pesticide. Dissolved, adsorbed, and vapor-phase concentrations in the soil are estimated by simultaneously considering the processes of pesticide uptake by plants, surface runoff, erosion, decay, volatilization, foliar wash-off, advection, dispersion, and retardation.

Uncertainty associated with each of these individual components adds to the overall uncertainty of the modeled concentrations. Equations in the model have not been shown to exert any directional bias. Model inputs from the required environmental degradation studies are chosen to represent the upper confidence bound of the mean, and are not expected to be exceeded in the environment 90% of the time. Mobility input values are selected to be representative of conditions in the open environment. Natural variation in soils adds to the uncertainty of modeled values. Factors such as application date, crop emergence date, and canopy cover can affect estimated concentrations. Ambient environmental factors, such as soil temperatures, sunlight intensity, antecedent soil moisture, and surface water temperatures may cause actual aquatic concentrations to differ from the modeled values..

6.1 Effects Assessment Uncertainties

6.1.1 Age Class and Sensitivity of Effects Thresholds

It is generally recognized that test organism age may have a significant impact on the observed sensitivity to a toxicant. For guideline tests, young (and theoretically more sensitive) organisms are used. Testing of juveniles may overestimate toxicity at older age classes for active ingredients of pesticides which act directly (without metabolic transformation) on the organism, because younger age classes often have not developed enzymatic systems associated with the detoxification of xenobiotics. When the available toxicity data provides a range of sensitivity information with respect to age class, the risk assessors use the most sensitive life-stage information as measures of effect.

6.2.2 Use of Surrogate Species Data

Currently, there are no FIFRA guideline toxicity tests for amphibians. Therefore, in accordance with EFED policy, data for the most sensitive freshwater fish are used as a surrogate for aquatic-phase amphibians such as the Barton Springs salamander. Available open literature information on metolachlor toxicity to aquatic-phase amphibians (African clawed frog) shows this species approximately is 3 to 4 times less sensitive than the freshwater fish endpoint EFED used in the assessment. Species sensitivity distribution data for amphibians indicates the range of sensitivity is similar to that of freshwater fish (Birge *et al.,* 2000). The African clawed frog appears to be less sensitive than some of the native species. Therefore, the endpoint based on freshwater fish ecotoxicity data is assumed to be protective and extrapolation of the risk conclusions from the most sensitive tested species to the Barton Springs salamander is more likely to overestimate the potential risks than to underestimate the potential risk. At the time of the assessment, it was not known where Barton Springs salamanders may fall in a species sensitivity distribution.

6.2.3 Extrapolation of Effects

Length of exposure and concurrent environmental stressors (*e.g,* urban expansion, habitat modification, decreased quantity and quality of water in Barton Springs, predators) will likely affect the response of the Barton Springs salamander to metolachlor. Because of the complexity of an organism's response to multiple stressors, the overall "direction" of the response is unknown. Additional environmental stressors may decrease or increase the sensitivity to the herbicide. Timing, peak concentration, and duration of exposure are critical in terms of evaluating effects, and these factors will vary both temporally and spatially within the action area. Overall, the effect of this variability may result in either an overestimation or underestimation of risk

6.2.4 Acute LOC Assumptions

The risk characterization section of this assessment includes an evaluation of the potential for individual effects. The individual effects probability associated with the acute RQ is based on the assumption that the dose-response curve fits a probit model. It uses the mean estimate of the slope and the LC_{50} to estimate the probability of individual effects.

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[12] Complete ECOTOX Reference List in Appendix F

Appendix A:

Brief Life History of the Barton Springs Salamander

Appendix B:

Summary of Ecological Effects

Associated with Metolachlor

Appendix C:

Scenario Development for

Barton Springs Exposure Modeling

Appendix D:

Exposure Modeling

Appendix E:

RQ Methods and LOCs

Appendix F:

Open Literature References

Meeting ECOTOX and OPP Acceptability Criteria